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NUTRITION REVIEWS

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NUTRITION REVIEWS

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THE BEGINNINGS OF ESSENTIAL NUTRITION

From the earliest application of chemical methods to the study of nutrition the two most important objectives were to learn what a diet must provide to supply the physiologic needs of the body, and to find analytic procedures for assessing the nutritive values of foods. At the end of a century of experimenting, knowledge pertaining to these purposes was very imperfect.

In a prefatory chapter (*Ann. Rev. Biochem.* **22,** *1–16 (1953)*) I gave an account of how I came to know of the studies of Lunin, Socin, and others, who between 1879 and 1906 experimented with diets containing only isolated proteins, carbohydrates, fats and inorganic salts, and found them to be inadequate for the survival of mice. It was these failures that convinced me that the most fundamental problem in nutrition was inquiry concerning their cause. Thus convinced, late in 1907 I started what was probably the first rat colony for making comprehensive investigations of the problem. At that time my speculations did not go beyond views already suggested and tested, *viz.*, that provision of certain organic substances such as phosphoproteins, phospholipids, nucleic acid, or organic compounds of iron was necessary to make a "purified" diet complete.

In 1912 the only outstanding new viewpoint which had been expressed in textbooks on nutrition within twenty years was the change of emphasis from protein to amino acids. For half a century protein, estimated by multiplying the nitrogen content of a food by a factor, had been assumed to have the same nutritive value regardless of the source. This concept had to be abandoned because: (1) Nasse showed that the amounts of ammonia yielded by several proteins after hydrolysis differed greatly, (2) Kossel showed that similar variations were found in the yields of the basic amino acids, and (3) E. Fischer showed that proteins differed over a wide range in their yields of certain monoamino acids. The conclusion that proteins must differ in their nutritive values was inevitable.

T. B. Osborne was an outstanding contributor to knowledge of proteins in his generation. He showed that in each of thirty-two kinds of plant seeds which he studied there were several kinds of proteins with distinct individualities, and that none of these was duplicated even in such closely related cereals as wheat, rye and barley. He availed himself of both chemical and immunologic technics in his studies. The extent and thoroughness of his work attracted wide attention to his experimental data.

Among the first to observe the effects of deprivation of an amino acid were E. G. Willcock and F. G. Hopkins (*J. Physiol.* **35,** *88 (1906)*). They used a "purified" diet in which zein, which lacks tryptophan, supplied the protein. Without tryptophan mice survived, on an average, fourteen days. With a supplement of this amino acid the average survival was twenty-eight days. Willcock and Hopkins did not refer to the earlier experiments of Lunin, Socin, and several others who had previously experimented with "purified" diets and it is doubtful whether they knew about them.

The epoch-making experiments of Eijkman and of Grijns, begun in 1896 (C. Eijkman, *Geneesk. Tijdschr. v. Ned.-Ind.* **36,** *214 (1896)*; G. Grijns, *Ibid.* **41,** *3 (1901)*), in which they induced avian beriberi in fowls by a diet of polished rice, and prevented or cured it by feeding whole rice, or extracts of rice polishings, even when they were verified

with inmates of prisons by A. G. Vorderman (*Ibid.* **38,** *47* (*1898*)), did not immediately secure the recognition they deserved. Arguments about the cause of beriberi were not stilled until the publication of the studies of H. Fraser and A. T. Stanton (Studies, Institute for Medical Research, Federated Malay States, Kelly and Walsh, Singapore, Publishers (1907–15)). The production of experimental scurvy in guinea pigs by A. Holst (*J. Hyg.* **7,** *619* (*1907*)), and demonstration of its cause and prevention, did not lead to extensive prevention of scurvy in infants until A. F. Hess and L. J. Unger (*J. Am. Med. Assn.* **69,** *1583* (*1917*)) applied the method in pediatric practice.

In 1906 F. G. Hopkins delivered a lecture (*The Analyst* **31,** *385* (*1906*)) in which he expressed the belief that there were "accessory food substances," deficiency of which caused disease. He mentioned rickets and scurvy, but not beriberi, and suggested that there was evidence that lecithin was essential in the diet.

None of the new ideas was mentioned by Voit, Rubner, Atwater, Lusk, Chittenden, or others, previous to about 1915. Their teachings were the bases of courses in human and animal nutrition. Protein and energy requirements, chemical analyses of foods, respiration and digestion, and nitrogen balance experiments were the subjects discussed.

That an era had come to a close is shown by the following statement by H. P. Armsby (*Exp. Sta. Rec.* **18,** *508–21* (*1907*)): ". . . . we have come to question some of our feeding standards. Some we have modified, and we hold them more flexibly than we once did, but protein, carbohydrates and fats are still the nutritional trinity. Our theory of nutrition has become traditional, and has little pedagogic value, and little inspiration for the investigator. As a natural result it is more or less out of touch with practice, while our experiments, upon the practical side of the subject, have been marking time." More emphasis was placed by leading investigators on the relative merits of low- and high-protein diets than on the evidence that unidentified nutrients were important. Human and domestic animal experiments still held the field in experimental studies.

For the reason that introduction of the rat as the animal most useful for comprehensive studies of nutrition problems was so important in the history of the study of foods and the nutritive needs of humans and animals, I record the circumstances which attended the setting up of my colony.

In 1906 E. B. Hart succeeded S. M. Babcock as professor of agricultural chemistry at the University of Wisconsin. The latter suggested an experiment to test the theory that nutrients, as estimated by chemical methods, were equivalent when obtained from different sources. Heifers less than half grown were to be restricted throughout the remainder of their growth period, and in adult life, to nutrients derived from single plant sources. The corn (maize), oat and wheat plants were to be compared. Parts of the plant—seed, leaf, stem, etc.—were to be combined in such proportions that the three rations showed the same chemical composition. It was not long before the wheat-fed group showed signs of malnutrition. They became blind. Later they were to deliver premature, stillborn calves. The corn- and oat-fed animals were in much better nutritional status.

I was studying under Professor Mendel when, in the spring of 1907, Professor Hart inquired of him about a young man with biochemical training, who would study the cause of the inferiority of the wheat ration, and the factors involved in the entire experiment. On Mendel's recommendation I accepted a position on Hart's staff, and devoted four years to chemical work on the problem.

In the prefatory chapter referred to (*loc. cit.*), I gave some details of the progress of the study, and of my loss of confidence that I was making progress toward its solution; my reading brought to my attention several failures which others had recorded in efforts

to nourish mice with "purified" food mixtures. This also increased my enthusiasm for inquiring into the causes for these failures of diets, the latter being complete so far as chemical analysis could show.

When I laid before Hart my ideas about using small animals, preferably rats, for learning the nature of the defects of "purified" food mixtures, he questioned the plan. I should, he said, devote my time to our novel and much lauded project with the cows, and not set out on some other enterprise. But when I told Dr. Babcock about my plan, he responded enthusiastically, and without delay took me to the office of Dean H. L. Russell to lay the project before him. When I had related my thoughts about the merits of the problem and of using rats as experimental animals, he dismissed the suggestions as unacceptable for two reasons: in an experiment station we should devote our efforts to the study of farm animals; the rat was a pest, and if it were to get abroad that we were using federal and state funds for feeding rats we should be subjected to severe criticism. But Babcock was undaunted by the Dean's adverse decision. He told Hart that he wanted me to have permission to carry out my plan, and on his insistence my chief gave me permission to do so. So far as I know nothing was said about the matter to the Dean and the undertaking was not made a formal project, a procedure which I am sure is occasionally made good use of today.

The status of the rat colony in the Experiment Station can be judged by the following events: Having tried using wild rats, trapped in the horse barn, I concluded that domestic rats would be better suited to my experiments. I bought a dozen albinos from a pet stock dealer and paid for them myself.

Elvehjem, in his biographic memoir (*J. Nutrition* **51,** *3, see esp. p. 9 (1953)*) said: "It is told that he [Hart] was instrumental in helping Doctor McCollum differentiate vitamin A from vitamin B because he felt that McCollum was spending too much money for the purchase of the sugar—lactose. In the early studies McCollum used as part of the ration for experimental animals the milk sugar lactose, but it was rather expensive and Professor Hart suggested that he use a cheaper form of carbohydrate. When this was done the ration was not only deficient in vitamin A but also in the water-soluble vitamin complex. The reason for this was the fact that lactose as manufactured in those early days carried with it considerable quantities of the B vitamins which were present in the original milk." Spending money for supplies for the rat colony in the early years irritated Hart. On no other matters than the rat colony did we have any conflict of opinions. I have always looked back on my ten years as a member of Professor Hart's staff as happy ones, rich in rewarding experiences.

In compounding the wheat-plant ration for cows a blunder was committed which I should have at once detected because of my experience with farm practice in handling wheat. It was the almost complete omission of the leaf portion of wheat from the ration. The only plots of wheat on the Station farm were grown for selecting superior varieties for planting in Wisconsin, and all wheat products fed the cows were purchased. Farmers near Madison sold the wheat straw which we used. In threshing, the leaves of wheat are beaten by the machine into small fragments, which are mostly left behind when straw is thrown upon a wagon with a pitchfork, hence what the farmers delivered to us was what we asked for—straw. The corn and oats which we used for the other groups were grown and properly handled on the Station farm, so those animals fed the latter grains received their full quota of well-cured leaves.

Not until 1916, in experiments on rats (E. V. McCollum, N. Simmonds, and W. Pitz, *Proc. Soc. Exp. Biol. Med.* **13,** *129 (1916)*), did I become aware that the leaf of a plant is far superior to the seed as a source of a number of nutrients, and that whereas

no combinations of seeds made a good diet, mixtures of a leaf and a seed nourished young rats well through growth and maturity. In the experiment with the heifers we were dealing with the effects of multiple deficiencies, principally those of calcium and vitamin A. No amount of inquiry by chemical methods then known would have revealed what was wrong. Of these facts we were unaware in 1911 when the results of a four-year study of the cows was published (E. B. Hart, McCollum, H. Steenbock, and G. C. Humphrey, *Res. Bull. No. 17, Wisc. Agr. Exp. Sta., 1911*).

My early experiments with rats fed "purified" diets were not well planned. The amino acids and the organic phosphorus compounds were dominant in my thinking and planning, so I used different proteins, singly and in combinations. Some contained phosphorus, others were phosphorus-free. Starch, lactose, sucrose, and fats from several sources were included in the diets tested. I was strongly influenced by Pavlov's writings on the psychic factors in digestion and so gave much thought to improving the palatability of my insipid food mixtures. "Impurities" in the materials which composed the diets, especially in the milk sugar, together with the beneficial effects of coprophagy, which I did not suspect as a source of error, vitiated many of my experiments.

I was not much impressed by Hopkins' hypothetic "accessory food substances," and did not become acquainted with the discovery of Eijkman and Grijns until I read the illuminating Cartwright Prize Essay of E. B. Vedder ("Beriberi", *William Wood and Company, New York (1913)*), and C. Funk's "Die Vitamine" (*Bergmann, Wiesbaden (1913)*). These books broadened my understanding.

The first important discovery which we made with the rat colony was that fats were not, as had been previously believed, sources of energy only, but that some contained an unsuspected nutrient, later known as vitamin A. The second was the demonstration that three known substances, *viz.*, a protein (casein) to supply additional amino acids, a calcium salt, and a fat containing the newly found fat-soluble vitamin, added to a cereal grain diet made it complete nutritionally. Any one of these supplements alone improved it somewhat, two improved it still more, and all three together made it nutritionally complete. This type of experiment I called the *biological method* for the analysis of a food. It yielded much more information about the nutrients in a food than did the chemical analysis of that time.

In 1915 Miss Davis and I found that while whole rice was made complete nutritionally by the three supplements named, polished rice required these and in addition something which was contained in water extracts of wheat germs, yeast, or boiled egg yolk. This observation clearly differentiated water- and fat-soluble nutrients of unknown nature (E. V. McCollum and M. Davis, *J. Biol. Chem.* **23,** *181 (1915)*). It was our belief that the water-soluble essential was identical with Eijkman's antiberiberi factor.

In 1909, after the triumphs of Dr. Osborne over a twenty-year period in adding to knowledge of proteins, he and Mendel established their rat colony, the second of its kind, for the purpose of testing with growing animals the relative values of individual proteins in Osborne's unique collection. During the early years of my work at the University of Wisconsin, I wrote to Dr. Mendel and kept him informed of what I was doing. He thought well of the cow experiment, and commended my project for studying the "purified" food problem.

He and Osborne first tried feeding a purified protein with starch, lard and a salt mixture. Their young rats failed promptly and died. They then solved admirably their specific problem of comparing individual proteins by using 28 per cent of deproteinated whey, evaporated to dryness. This they called "protein-free milk." This fur-

nished sufficient unknown "accessory food factors" to make their basal diet complete if the amino acid yield of the protein was such as to support well-being in the rat.

They next prepared "artificial protein-free milk" by combining milk sugar with inorganic salts to imitate the mineral content of whey. This preparation, with a purified protein, supported for a time fairly satisfactory growth in young rats. That they did not at that time believe any "accessory factors" to be essential for normal nutrition is shown by a statement by Mendel made before the XVth International Congress on Hygiene and Demography (*Washington, D.C., September 1912*):

"Latterly Osborne and I have further succeeded in imitating the inorganic makeup of the natural 'protein-free milk' by uniting the various ions shown by analysis to be therein, into an artificial salt mixture. This too has proved to be a reasonably successful adjuvant; and the results furnish, so far as we are aware, the first published records of successful feeding experiments in which prolonged growth has been induced by carefully purified, isolated foodstuffs and an artificial salt mixture. By prolonged growth we refer to the capacity of animals to triple or quadruple their weight." In the same paper (*p. 6*) he added: "The simplest food mixtures with which characteristic growth curves have been obtained have been made up of only one protein, starch, and sugar, and the artificial salt mixture, i.e., they have been fat- and presumably lipid-free."

They deceived themselves, as I had deceived myself, as to the purity of some of the ingredients of their diets. Every claim made in the above quotations had to be abandoned as the result of another year of experimenting. First, they found that a supplement of butter (containing whey) benefited failing animals. Then, five months after our announcement that the ether-soluble matter of butter contained an essential nutrient, they confirmed our observation on this point.

From that time forward investigators took into account quality in proteins, the need for both water- and fat-soluble unidentified nutrients, and sensitiveness of animals to deficiency of calcium. From 1916 the study of nutritive factors in foods, and their relative abundance in common articles of diet, characterized many studies. Fruitful investigations were initiated as the result of Holst's discovery that guinea pigs were susceptible to scurvy, to which the rat is immune.

From the point at which this account ends, studies of foods and nutrition increased rapidly. Twenty-five years of inquiry by some hundreds of investigators revealed that four fat-soluble vitamins exist, and that what, in 1915, we regarded as a single water-soluble essential consisted of a complex of a dozen or more components, deficiency of each of which caused a specific type of malnutrition. The roles of individual amino acids, of fatty acids, of essential inorganic elements, and of vitamins, were examined experimentally by methods based on the pioneering work which marked the transition from the old to the new in this field of inquiry.

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FAMILIAL HYPOGLYCEMIA PRECIPITATED BY AMINO ACIDS

Hypoglycemia or low blood glucose can lead to a great variety of symptoms in man, ranging from increased hunger and mild tremulousness to convulsions, coma and even death. The multiple etiologies of hypoglycemia have been well classified by J. W. Conn and H. S. Seltzer (*Am. J. Med.* **19,** *460 (1955)*).

The first major category of causes of hypoglycemia is called "organic" and includes tumors of the pancreas, liver disease, pituitary and adrenal insufficiency, rare abdominal fibromas and sarcomas and certain destructive lesions involving the hypothalamus and brain stem. The second major category is considered "functional" and includes those cases without recognized anatomic lesion but explainable on the basis of unusual somatic function. The most important clinical syndrome in this category is the common functional hypoglycemia found in young adults who either exhibit neurotic tendencies or manifest instability of the autonomic nervous system. Characteristically these patients have symptoms appearing two to three hours after meals and not after an overnight fast or even after a more prolonged fast. J. W. Conn has found (*J. Clin. Invest.* **15,** *673 (1936)*) that a high protein diet is effective in the treatment of these subjects. Protein rather than carbohydrate is increased in the diet because the former allows a slower absorption and a slower conversion to carbohydrate and thus avoids the extreme fluctuations of blood sugar which appear to be detrimental in these individuals. Conn's observations have been confirmed by other investigators and the dietary therapy of functional hypoglycemia has become generally accepted.

There is another type of functional hypoglycemia occurring in infancy which is distinctly different from that found in young adults. The infantile hypoglycemia appears most commonly during the first year of life but instances of the disorder may not be recognized until as late as 5 years of age. Hypoglycemia occurs both during fasting and following meals, and results of tests of carbohydrate metabolism are indistinguishable from those of patients with tumors of the islets of Langerhans of the pancreas. Exploration of the pancreas has failed to reveal pancreatic disease in the majority of cases, although at one time deficiency of alpha cells was believed to be a common finding (I. McQuarrie, E. T. Bell, B. Zimmerman, and W. S. Wright, *Fed. Proc.* **9,** *337 (1950)*). A strong familial tendency has been observed by a number of workers.

Dietary therapy of the hypoglycemia of infancy has been much less successful than it has been in adult cases of hypoglycemia. N. B. Talbot, J. D. Crawford, and C. C. Bailey (*Pediatrics* **1,** *337 (1948)*) described the response of one patient to a high protein, low carbohydrate feeding: "... during this period, the fasting blood sugar concentrations fell and the patient became so much worse that it became necessary to institute intravenous dextrose therapy." A similar experience was had by W. A. Cochrane, W. W. Payne, M. J. Simpkiss, and L. I. Woolf (*J. Clin. Invest.* **35,** *411 (1956)*) and it led them to consider the effect of protein on the blood sugar of certain patients with familial hypoglycemia. Observations are presented on 3 infants who developed severe hypoglycemia with convulsions early in life, and on the father of 2 of the children who gave a history suggesting infantile hypoglycemia.

The plasma insulin-like activity of one infant was said to be normal; in another increased levels were observed. The difficulty of this assay makes interpretation hazardous. Glucose tolerance tests demonstrated fasting hypoglycemia with a relatively normal-shaped curve and a return to hypoglycemic levels in two to three hours. Epinephrine and glucagon produced a normal hyperglycemic response, indicating that there was not a defect in the conversion of glycogen to glucose as occurs in glycogen storage disease. Administration of ACTH or cortisone produced only a slight increase in blood sugar but was usually successful in controlling convulsive phenomena.

The most interesting observations of Cochrane and his co-workers concern the effects of various protein and amino acid feedings on blood sugar. When 1.5 g. of casein per kilogram of body weight were administered orally to infants with this

condition, a prompt fall in blood sugar occurred. The administration of comparable amounts of casein to normal infants and to another individual with hypoglycemia presumably of a different type did not result in a significant alteration of the blood sugar. In contrast to casein, gelatin was found to have only a slight hypoglycemic effect.

It was suspected that the difference in the ability of the two proteins to produce hypoglycemia could be attributed to differences in amino acid composition. Tyrosine, an amino acid found in much higher concentrations in casein than in gelatin, did not have a hypoglycemic action. Leucine (0.08 to 0.23 g. per kilogram of body weight), on the other hand, produced a significant drop in the blood sugar in all 4 subjects with this condition studied whereas little change in the blood sugar was produced in normal subjects. Isovalerate, a metabolite of leucine, was hypoglycemic in a single instance of the syndrome. It was suggested by the authors that the hypoglycemic effect of casein might be attributed, in part at least, to an increased concentration of leucine. This amino acid or its metabolite, isovalerate, may either increase the action of insulin or augment its production or release by the pancreas. Verification of this suggestion will require much additional work.

The observations of Cochrane and his co-workers are of both practical and theoretic interest. Feeding high protein diets, particularly if the protein is casein, to infants suffering from hypoglycemia may be harmful and should be done with caution. On the theoretic side, one may wonder whether the failure of one patient with infantile hypoglycemia to develop hypoglycemia upon casein feeding indicates that there are two metabolic types of this syndrome, one with and one without increased hypoglycemia due to casein or leucine. To speculate further, familial hypoglycemia may represent an inborn error of metabolism one feature of which is an abnormal metabolism of leucine. The observed susceptibility to hypoglycemia following leucine administration might, however, be attributed to the low initial blood sugar and a relative deficiency of glucose-forming mechanisms. Understanding of the underlying mechanism involved in infantile hypoglycemia awaits further clarification.

KWASHIORKOR IN UGANDA AND COONOOR

The syndrome in children characterized chiefly by edema, stunted growth, skin and hair changes, and a fatty liver has been reported from many of the less well developed regions of the world. Minor variations in the clinical picture and a multiplicity of names tended, for a time, to confuse the fundamental similarities of the condition in different regions. Although it is now believed that protein malnutrition is the common basis for this syndrome, and kwashiorkor is gradually being adopted as the common name for the disease, the causes of the variations in the clinical picture and their significance remain obscure. Comparisons made in different areas should contribute to a more complete understanding of the disease and the etiologic factors involved. C. Gopalan (*J. Trop. Pediat.* **1,** *206 (1956)*), who has studied the disease extensively in India, has had an opportunity to visit workers and clinics in Uganda and has made a comparison of some of the similarities and differences seen in these two areas.

Although African diets vary from area to area more than those in many parts of India, there are apparently three major dietary groups in Uganda. In Baganda the principal food is plantain (banana-like fruit), and considerable amounts of sweet potato, peanuts, millet, beans and maize are eaten. In nearby Ruanda the staples are similar, but

more sweet potato and maize are eaten. Gopalan reports that some of the worst cases of kwashiorkor and some cases of nutritional edema in adults are seen in this area. The Nilotic tribes to the north were previously mainly dependent upon millet, beans and peas for their diet, but in recent years cassava production has expanded and kwashiorkor is frequently seen.

The African infants are generally breast-fed until the twelfth or eighteenth month of age but receive supplementary feeding from the fifth month onward. This is usually no more than small quantities of plantain and sweet potato. Although dietary histories of young children are notoriously difficult to obtain, various estimates suggest that many of the children from 1 to 4 years of age are getting about 20 g. of protein or less. Thus, the diets are similar to those in Coonoor where supplementary feedings, usually of rice gruel and occasional vegetables, are started about the sixth month. The diets are deficient all around, but believed to be primarily lacking in protein. The author points out that the precise significance of these findings is very difficult to assess. The workers in Africa as well as in India have not been able to show a direct relationship between the protein intake and the development of kwashiorkor. It is not only the poor children who develop the disease, and this fact leads the Indian workers to suggest that attention must be paid not only to the diet, but also to the duration of the deficiency, and other factors such as infections, infestations, etc.

Psychologic factors may be important. Many of the African infants who contract the disease have been separated from their mothers for various reasons. The child may resent this and express his feelings by refusal to eat. Thus, the anorexia which is an important feature of kwashiorkor may either be a cause or a result of the disease. The habit of sending the children to the grandmother or other relative does not prevail in India, but " 'the child can be effectively separated from the mother without going far from her side.' " Since kwashiorkor is often associated with weaning, many times due to the presence of a new baby in the family, the psychologic aspects of the disease clearly need further study. It is important to remember, however, as emphasized by M. Geber and R. F. A. Dean (*Courrier* **6,** *3* (*1956*)) that protein malnutrition itself is strikingly characterized by mental depression and psychologic impairment.

Infestations and infections may affect the onset and course of the disease, especially with respect to food utilization and, in turn, the deficiency may create greater susceptibility to infections (N. S. Scrimshaw, M. Behar, C. Pérez, and F. Viteri, *Pediatrics* **16,** *378* (*1955*)). In one area M. D. Thompson (Gopalan, *loc. cit.*) found only 19 out of 164 children with kwashiorkor who did not show signs of infection. Practically every infant who developed the disease with a seemingly adequate protein intake showed evidence of a febrile illness or gastrointestinal upset. The anorexia induced by such illnesses may be an important factor. Similar relationships may exist with regard to intestinal parasites. In Ruanda and Coonoor heavy infestations of *Ascaris* appear to be the rule, whereas in Baganda hookworm is much more prevalent. J. N. P. Davies (Gopalan, *loc. cit.*) has indicated that the age periods when the diets become low in protein and the risk of hookworm begins are similar.

The possibility that malaria might be an important etiologic factor was also considered. There appears to be no unanimity of opinion. However, malaria is absent from areas, such as Coonoor, where kwashiorkor is prevalent. Nonetheless, it seems likely that if the malaria is sufficiently severe to cause vomiting and diarrhea, the development of kwashiorkor would be enhanced. Attitudes toward blood transfusions and iron therapy in the treatment of kwashiorkor would certainly be different depending upon the presence or absence of hookworm and malaria.

In commenting upon the causes of the poor diets, Gopalan indicates that in India dire poverty and sheer inability to get appropriate food is mainly responsible. On the other hand, his impression is that in Uganda faulty dietary habits resulting from tribal traditions and disproportionate family budgeting may be much more important factors. Even the poorest hut in Uganda usually had some land available for food production, but this was most commonly used for growing plantain and sweet potato. Thus, there appears more promise for education in the prevention of kwashiorkor in Uganda than in India, although ignorance contributes to the disease in both areas.

Various similarities and differences in the clinical features are discussed. The age incidence is similar in Africa and India, with a peak between the first and third years of life. However, after 3 years of age there is a considerably higher incidence in the Indian children. Although completely adequate data are not available, it would appear that the pattern of weight increase in African and poor Indian children is similar. A comparison of the weights of children with kwashiorkor shows the Indian children to be more underweight than the African children. This may indicate that the degree of protein restriction is greater in India. This impression is also gained by clinical examination.

Skeletal growth in normal Indian children is similar to that in American children whereas the skeletal growth of those with kwashiorkor lags behind. There is apparently a divergence of opinion as to whether this is true in Africa.

The hair of the African child seems to be extraordinarily susceptible to changes in color and texture. The average infant observed by Gopalan in Africa apparently only had jet black hair for about two months, and all shades of discoloration were observed afterward. In some children with kwashiorkor even extensive treatment with high protein diets may not cause a change in color of the hair. On the other hand, in India, where the degree of deficiency would certainly appear to be as severe, only 48 per cent of the cases showed classic hair changes.

Anorexia is a notable feature of kwashiorkor in all areas, although "cases of established kwashiorkor were sometimes seen with good appetite." The question of whether this is primarily a cause or a result has been mentioned above. Diarrhea is also common in both India and Africa and quite often may precede and possibly precipitate the disease. Steatorrhea, too, is a common feature in most cases. R. F. A. Dean (Gopalan, *loc. cit.*) has observed that whereas animal fats are usually poorly tolerated, vegetable fats may not be aggravating.

Hepatic enlargement was found in only 10 per cent of the Indian children studied and the incidence was also low in Uganda. This is a much lower incidence than has been reported in Jamaica and Indonesia. Since the size of the liver is dependent upon the degree of atrophy and the amount of fatty infiltration, and since there was severe fatty infiltration in children seen in both Coonoor and Uganda, it is concluded that in these areas there is relatively more atrophy than in Jamaica and Indonesia.

Xerosis or keratomalacia was found in 36 per cent of the Indian children and a muddy discoloration of the conjunctiva was almost an invariable feature of the disease. By contrast, the incidence of vitamin A deficiency in Uganda was apparently negligible, and clear white conjunctiva were the rule. This difference is probably due to the carotene in sweet potatoes commonly eaten by the African children. Circumcorneal pigmentation is seen in both regions and is apparently associated with dark-skinned people, and not related to vitamin A deficiency as was originally thought.

In a follow-up of 100 cases in Coonoor, 38 per cent were found to have had relapses from which they had either recovered or died. The evidence suggests that relapses may be less common in Uganda, although

this may depend upon the region. The author suggests that the individuals who do not relapse when sent back to their original environment may be of more interest. This would again suggest that a straightforward relationship between diet and kwashiorkor need not be expected.

Although skim milk or skim milk supplemented with casein has been most widely used in diet treatment, there is work under way in both Africa and India upon the use of vegetable proteins of various kinds. Evidence is accumulating that various food mixtures may prove entirely satisfactory. Such studies may prove to be a landmark in the struggle against this disease.

NITROGEN, FAT AND MINERAL METABOLISM IN GASTRECTOMIZED PATIENTS

Although man can get along without his stomach, the operation of total gastrectomy results in certain alterations in his metabolism. There have been several reports (*see, for example*, T. C. Everson, *Internat. Abstr. Surg.* **95,** *209* (*1952*) and W. D. Kelly, L. D. MacLean, J. F. Perry, and O. H. Wangensteen, *Surgery* **35,** *964* (*1954*)) of such alterations, and recently M. K. Schwartz, O. Bodansky, and H. T. Randall (*Am. J. Clin. Nutrition* **4,** *45, 51* (*1956*)) have reported a series of studies of nitrogen, fat and mineral metabolism in totally gastrectomized patients, and have discussed their findings in relation to previously published work along similar lines.

As regards nitrogen metabolism, 7 patients with total gastrectomies were studied for 15 three-day metabolic periods from four months to five years after operation. During these metabolic periods, determinations were made of fecal and urinary nitrogen excretion. Schwartz *et al.* point out that the nitrogen balance or protein intake can be expressed in a variety of ways, as grams (D. M. Hegsted, A. G. Tsongas, D. B. Abbott, and F. J. Stare, *J. Lab. Clin. Med.* **31,** *261* (*1946*)), grams per kilogram (E. P. Benditt, R. W. Wissler, and P. R. Cannon, *Tr. 17th Meeting, Josiah Macy Foundation, p. 11* (*1948*)), grams per square meter (J. B. Allison and J. A. Anderson, *J. Nutrition* **29,** *413* (*1945*)), or milligrams per basal calorie (M. Bricker, H. H. Mitchell, and G. M. Kinsman, *Ibid.* **30,** *269* (*1945*)). Schwartz *et al.* state that "the balance represents the difference between the intake and the total excretion and therefore is subject to the same absolute difference or variability as these terms and to a greater percentage variability." Thus, in their studies, the total nitrogen excretion in milligrams per kilogram of body weight was plotted against the intake expressed in similar units, the slope of any regression equation representing the fraction excreted per unit of intake, and one minus this fraction representing the utilization. Schwartz *et al.* further state that a true control group for their gastrectomized subjects would be normal persons who had been starved and were being repleted. A search of the literature revealed some suitable post-World War I data (H. von Hoesslin, *Arch. f. Hyg.* **88,** *147* (*1919*)), and similar data, obtained by J. Beattie, P. H. Herbert, and D. J. Bell (*Brit. J. Nutrition* **1,** *202* (*1947*)) after World War II. The results of both these normal metabolic studies were analyzed statistically by Schwartz *et al.* along the lines already mentioned, and compared with data obtained from the 7 gastrectomized patients. The results thus obtained indicated that in the gastrectomized patients fecal nitrogen excretion was significantly related to nitrogen intake, and that the amount of dietary nitrogen needed to prevent negative nitrogen balances was greater than in normal subjects.

As regards fat and mineral metabolism, Schwartz *et al.* studied the 7 gastrectomized patients discussed above, as well as 2 additional ones, a totally gastrectomized subject

without steatorrhea, and one with steatorrhea; in this latter case, the relationship between calcium and dietary fat was also studied. As in the nitrogen metabolism studies already referred to, data concerning fat and mineral metabolism in normal subjects were obtained from previously published reports in the literature. Eight of the 9 totally gastrectomized patients had a high excretion of fecal fat. The regression line representing the relationship between fat intake and excretion was significantly different from that in normal persons, and the average fecal fat excretion, 20.2 per cent of the intake, was significantly higher than that calculated from data for normal individuals. Finally, as regards minerals, Schwartz *et al.* found that the metabolism of calcium, phosphorus, magnesium and potassium, as represented by the regression equations relating the total excretion to the total intake, did not differ in the gastrectomized patients from that in normal controls.

The extensive use, for normal control purposes, made by Schwartz *et al.* of previously published reports by other workers is perhaps open to some criticism. For example, in their studies of nitrogen metabolism, the regression coefficient obtained by Schwartz *et al.* in their series of gastrectomized subjects differed significantly from that of the normal post-World War I series of von Hoesslin (*loc. cit.*) but not from that of the post-World War II series of Beattie *et al.* (*loc. cit.*). However, these studies of Schwartz *et al.* should prove of much interest and value, particularly to those who have to do with arranging suitable dietary regimens for totally gastrectomized patients.

WHY ARE THE THIN LEAN?

It is not an uncommon lay belief that the thin individual remains lean because of some innate physiologic peculiarity which sets him apart from the rest of men. It has been proposed that the thin man is wasteful in expenditure of the energy consumed, or inefficiently absorbs the nutrients in his food. The work of German nutritionists at the turn of the century has been quoted as supporting the above proposition. They found that an increased protein intake progressively increased the specific dynamic action. To E. Grafe ("Metabolic Diseases and Their Treatment," *Lea and Febiger, Philadelphia (1933)*), this loss of excess energy in the form of heat represented an important method whereby the body was maintained in weight equilibrium.

A. Gulick (*Am. J. Physiol.* **60,** *371 (1922)*), a thin individual himself, found that on a high caloric diet and a standard program of activity, he gained weight less readily when he was considerably heavier than usual (74 versus 62 kg.). This observation was suggestive evidence for the validity of the theory put forth by Grafe. Unfortunately, Gulick was not able to control his energy expenditure closely enough to permit an accurate caloric balance sheet.

R. Passmore, A. P. Meiklejohn, A. D. Dewar, and R. K. Thow (*Brit. J. Nutrition* **9,** *20 (1955)*) measured the energy expenditure of 3 lean young men by means of the Kofrányi-Michaelis apparatus (*Nutrition Review* **12,** *196 (1954)*). This portable respirometer measures the volume of expired air and simultaneously collects a proportionate sample for subsequent analysis. The subjects, confined to a hospital metabolic ward during the period of the observations, were permitted only limited activity in order to reduce their total caloric expenditure. To this end they were kept in bed until 1:30 p.m. each day. Every afternoon they walked a constant distance of about 5 miles. The speed was maintained by requiring them to walk certain fractions of the distance within a set time. After the exercise they were per-

mitted to sit in the hospital ward quietly reading until they went to bed at 8 p.m. Each man recorded, to the minute, the time spent in bed, walking, sitting, and such activities as washing, dressing, and other necessary functions.

The subjects were normal young men (ages 21 to 24 years) who had had no major illness in the preceding three years. They were from 82 to 86 per cent of normal weight when compared to standard height/weight tables (57, 59 and 62 kg. or 125, 129 and 136 pounds). The experiments were carried out on one subject in September and on the other 2 in March.

Each subject was maintained on the controlled program for either four or five days. During that time he required 2100 Calories to maintain his body weight. This was followed by ten to fourteen days during which the caloric intake was increased to nearly 4000 Calories with no change in activity.

There was no marked difference in the metabolic activity of the men during the two dietary periods. This was shown by the fact that the energy expenditure as measured at four-hour intervals throughout the day and night and during the walk was the same on the high and low caloric diets. While the men were reading, presumably after supper, the energy expenditure during the period of the high caloric diet was 1.7 Calories per minute, whereas it was only 1.3 during the low caloric diet.

Passmore and co-workers (*loc. cit.*) suggest that the extra energy produced in the evening while the men were reading was probably due to the specific dynamic action of protein. The increased caloric intake resulted in an increase in the protein consumed from about 80 g. to 130 g. per day. It is difficult to understand why the specific dynamic action should have been produced only in the evening. This might be apparent if the composition of the diets had been given. It is also a little difficult to determine exactly when this evening spurt in energy occurred. The investigators state in one place that the evening measurement was made at 6 o'clock, yet the table shows the values for 7 p.m. At that time supper was being served, according to the protocol.

Regardless of the exact time when the increased caloric expenditure occurred, the contribution of this excess energy amounted to from 6 to 16 per cent of the total excess caloric intake. These differences are regarded as being too small to be of any physiologic significance, especially since the metabolic rate at 11 p.m. was essentially the same for the two dietary periods.

Fecal analyses showed that the absorption of calories, fat and nitrogen on a percentage basis (93, 96 and 87 per cent respectively) was unchanged by the increase in daily intake from 2100 to 4000 Calories. According to this, none of the 3 young men was thin because of a poor absorptive capacity in the intestinal tract.

The validity of the caloric calculations is based on a balance sheet for each young man during the control period. At that time the caloric intake and expenditure agreed perfectly for 2 of the 3 men. For the third subject, the caloric expenditure was 11 per cent higher than his intake. All 3 subjects during this time maintained their weight.

This study offers the best available evidence indicating that lean individuals have no metabolic or digestive peculiarity which will account for their leanness. Since these subjects consumed without effort all of the excess food represented by the high caloric period (ten to fourteen days), the study further suggests that the low caloric intake of these men when they are on an ad libitum diet is not limited by the capacity of their stomachs.

The increased caloric intake resulted in an increase in body weight. The obvious conclusion from the above work is that the thin person just doesn't eat enough to bring his weight up to normal standards. There is, unfortunately, no answer as to why he doesn't eat more. And so the formula that might benefit those who have difficulty controlling weight remains as elusive as ever.

Although the present study is an impor-

tant contribution to the physiologic characterization of lean individuals, it is unfortunate that the study did not include an equal group of individuals of normal weight or slightly obese. Furthermore, one might wonder what would have happened had the men been maintained on the high caloric diet for a longer period of time. It frequently requires a month or more to establish equilibrium for many physiologic functions.

ESSENTIAL AMINO ACID REQUIREMENTS

The extensive work of W. C. Rose and his associates (*see Nutrition Reviews* **14,** *232 (1956)*) was the first to yield definitive figures for the essential amino acid requirements of adult man using diets in which the nitrogen was essentially all supplied by purified amino acids. Since all scientific information may be considered somewhat tentative until it has had independent confirmation in another laboratory, the work of R. M. Leverton and her associates using similar technics is important. A series of 5 papers by Leverton *et al.* (*J. Nutrition* **58,** *59, 83, 219, 341, 355 (1956)*) presents the results of studies over the past five years upon the minimum requirements of young women for threonine, valine, tryptophan, phenylalanine and leucine.

The studies were done with 35 healthy college girls from 19 to 35 years of age. After a preliminary period in which the subjects ate a weighed diet of ordinary food and adjusted to the experimental routine, they were given a diet composed largely of sugar, cornstarch, purified butter, corn oil and an amino acid mixture. Small amounts of fruit juice, fruits, carrots and lettuce were used to improve the palatability of the diet. These materials were analyzed by microbiologic methods to determine their amino acid content. All subjects received a mineral mixture, vitamin supplements and 5 ml. of a liver extract. A considerable portion of the calories was supplied by especially prepared wafers and puddings made from the cornstarch, sugar and fats.

In most of the studies the diet contained the eight essential amino acids required by man as well as arginine, histidine, tyrosine and cystine in the amounts found in 20 g. of egg protein. This supplied a total of 1.91 g. of nitrogen. The amount of the essential amino acid under test was, of course, varied. Additional nitrogen was supplied as glycine or diammonium citrate. The total nitrogen intake varied from 6.2 g. per day to 9.5 g. per day in the various studies, but during most of the study the higher level was supplied. The amino acids were generally given in lemonade in divided amounts so that 25 per cent was taken at breakfast, 37.5 per cent at lunch and 37.5 per cent at dinner.

The experimental periods on a given level of amino acid were ordinarily of six days or occasionally a few days longer. Carmine markers were given to delimit the fecal collections every seven days, or when the diet was changed. Complete twenty-four-hour collections of urine were made and the nitrogen content was determined within four hours. Creatinine was also determined. Nitrogen balance was the criterion of adequacy of the intake of an amino acid, and the authors consider that balance is achieved when the excretion in urine and feces is within 95 to 105 per cent of the intake.

Care was taken to assure that the subjects did not lose or gain weight. Each subject was weighed daily and the caloric intakes were adjusted as seemed necessary. The calorie intakes varied from 35 to 54 Calories per kilogram of body weight, with an average intake of 2500 Calories or 42 Calories per kilogram.

In the studies on threonine, the intake of threonine was varied from zero to 735 mg.

of L-isomer per day. There appears to be a reasonably linear relationship between the amount administered and the degree of nitrogen balance, up to the level which produces nitrogen equilibrium. There was a great difference in the response of individual subjects. Some were in the zone of equilibrium (excretion = 95 to 105 per cent of intake) at 103 mg. per day, while others were not until the intake was 305 mg. per day. The causes of such variation are not apparent and each subject was not necessarily investigated systematically at all levels tested. All subjects were in the zone of equilibrium on an intake of 305 mg. per day. The authors suggest this level as the minimum requirement. However, an inspection of the data presented shows that at this level the mean balance of the group was slightly negative. An intake of 400 mg. per day produced a slight positive balance and there was no significant improvement in nitrogen retention at higher levels. This latter value may be a safer minimum estimate.

In the studies on valine, levels of 375, 465, 650 and 1560 mg. of L-valine were used. The mean balance was linearly related to the intake at the three lower levels of intake and was slightly positive with an intake of 650 mg. per day. All subjects were within the "zone of balance" with this amount and this level is suggested as a minimum requirement.

The investigation of the tryptophan requirement involved 8 subjects and intakes of 63, 82, 120, 157 and 307 mg. of L-tryptophan were used. All subjects were in the "zone of equilibrium" on an intake of 157 mg. per day, although the mean balance was slightly negative. One-hundred and sixty mg. per day is suggested as the minimum requirement.

The phenylalanine requirement was more complex because of the role of tyrosine. When the diet contained 900 mg. of tyrosine, 220 mg. of L-phenylalanine was sufficient to maintain balance in the group. When no tyrosine was included in the diet, increasing the levels of phenylalanine from 120 to 650 mg. per day caused no improvement in nitrogen balance. This was the highest level studied in the absence of tyrosine. It is surprising that no effect of phenylalanine could be demonstrated, but higher levels would be expected to produce a positive balance. Rose *et al.* found the minimum requirement in the absence of tyrosine to vary between 0.8 and 1.10 g. per day.

The effect of tyrosine was then studied at two different levels of phenylalanine. When 220 mg. of phenylalanine was included in the amino acid mixture, the mean tyrosine requirement was estimated as 900 mg. per day, although the next lowest level studied was 450 mg. per day. One subject was in strong negative balance on this level. When 420 mg. of phenylalanine was present in the diet, balance was achieved at 450 mg. of tyrosine. These studies demonstrate the expected interrelationship between tyrosine and phenylalanine but fail to give a clear picture of the ability of tyrosine to replace phenylalanine. An intake of 220 mg. of phenylalanine is suggested as the minimum requirement in the presence of adequate tyrosine, 900 mg. or more of the latter. The lowest level of the two combined which produced balance was 450 mg. of each, or a combined intake of 900 mg. per day. W. C. Rose (*see* "Recommended Dietary Allowances," *p. 8, National Academy of Sciences—National Research Council Publication 302, Washington, D.C. (1953)*) has estimated that from 25 to 30 per cent of the total phenylalanine-tyrosine requirement must be supplied by phenylalanine. If 220 mg. represents 25 to 30 per cent of the requirement, the total need would be of the order of 800 mg. per day.

In the final study the leucine requirement was estimated. In agreement with the work of Rose *et al.* (*see Nutrition Reviews, loc. cit.*), considerably more variation between subjects was noted with this amino acid than with the others studied. However, all

subjects were within the zone of balance at an intake of 620 mg. per day and no further improvement was noted at higher levels of intake. Several of the subjects were in negative balance at the next lowest level of intake studied, which was 480 mg. per day.

No specific symptoms attributable to amino acid deficiencies were noted, although occasional nausea, vomiting and gastrointestinal upsets may have been associated with the amino acid-low diets. Rose *et al.* (*see Nutrition Reviews* **13,** *71 (1955)*) have pointed out that about 25 per cent more calories were required by their subjects to maintain nitrogen balance on the amino acid diets than when intact protein was fed. If this was true in these studies with women subjects, it was much less apparent. The average intake of 2500 Calories does not appear exceptionally high.

It will be apparent that the values estimated by Leverton and her colleagues as the minimum requirements for young women are considerably below the estimates made for young men by Rose *et al.* (*see Nutrition Reviews* **14,** *232 (1956)*), but in all cases they fall within the range of values estimated by Rose's group as the requirement of different subjects. Rose *et al.* determined the minimal daily intake which would maintain a slight positive balance in each subject and chose the highest observed value for all of the subjects rather than the average as the "tentative minimal requirement." On the other hand, Leverton *et al.* have determined the average amount which would produce balance (excretion in urine and feces not over 105 per cent of the intake) in the group studied. These levels would have been considered insufficient by Rose's criteria. It may be argued which procedure is the better of the two. Leverton's approach is more in line with the procedures followed in studies upon vitamin requirements and more defensible statistically. On the other hand, when few subjects are being studied, it is undoubtedly better to err on the side of adequacy rather than the reverse.

Although there is so much variation in individual response that no clear relationship to weight is apparent, it is likely that the requirements of women would be somewhat lower than those of men. A further difference would be expected if the amino acid requirements are related to basal metabolism or lean body mass. Considering these differences in the subjects studied and the differences in interpretation of the results, it is clear that this work essentially confirms the results of Rose *et al.* Regardless of the mode of interpretation, the surprising findings are the very low requirements for the essential amino acids. Considerably less than 20 g. of high quality protein will meet the minimal requirements of young adults for nitrogen equilibrium as defined by these investigators.

These studies by Rose, Leverton, and their co-workers have established a solid starting point for the investigation of amino acid requirements of adults living under more stressful conditions than those to which American college students are exposed. Premature conclusions should not be drawn as to the application of these amino acid requirements data to individuals with different dietary histories and protein stores, living under other conditions of climate, work, exercise and exposure to infections or parasites. Obviously they are not to be applied to individuals whose anabolic needs are high, such as children, pregnant and lactating women, the chronically ill and convalescents.

W. C. Rose, G. F. Lambert, and M. J. Coon (*J. Biol. Chem.* **211,** *815 (1954)*) suggest doubling the minimum requirement to define a safe intake. They state further that "a safe intake is not to be confused with an 'optimum intake' which undoubtedly is still higher, but for which no direct measurement is available." This appears to be a reasonable position in the light of present knowledge, in that current estimates of amino acid requirements necessarily, because good technics of measurement are not yet

available, do not take account of the possible sparing action of body protein stores on requirements or allow for losses of nitrogen from the skin.

That body protein stores may exert a sparing action on amino acids is suggested by the study of E. S. Nasset and V. H. Gatewood (*J. Nutrition* **53,** *163 (1954)*) on the histidine requirement of the adult rat. They observed that a histidine intake adequate for nitrogen equilibrium was not necessarily adequate for maintenance of hemoglobin level, indicating that part of the histidine need of the animal could be supplied by catabolism of this protein.

M. Bricker, H. H. Mitchell, and G. M. Kinsman (*J. Nutrition* **30,** *269 (1945)*) estimate nitrogen losses in the perspiration of adult man to range from 0.40 to 3.74 g. daily, depending on whether sweating is minimal or profuse. The difference corresponds to 21 g. protein per day (N × 6.25). Additional nitrogen is lost in the constant sloughing of epidermis. Small amounts of nitrogen may be stored in hair and nails and in other tissues that change dimension with age. The loss of nitrogen in perspiration would appear to be the most important variable and could affect protein needs substantially. It is known that in maize-eating populations frank pellagra, accompanied by loss of body nitrogen, manifests itself particularly in the spring and summer months, when work and temperature combine to cause more profuse sweating (J. Gillman and T. Gillman, "Perspectives in Human Malnutrition," *p. 56, Grune and Stratton, New York (1951)*). The Gillmans report that the usual maize diet of the Bantu is adequate for convalescence of persons with pellagra if they are kept at rest in a hospital.

"Safe intakes" of amino acids and protein, therefore, could well be those which provide apparent storage of perhaps 0.5 to 1.0 g. of nitrogen daily under conditions of minimal sweating and of larger amounts under conditions of high temperature or humidity and hard physical labor. "Optimum intakes" would presumably be those which provide adequate labile protein stores in the body. J. B. Allison (*Fed. Proc.* **10,** *676 (1951)*), in work with adult dogs, has shown that labile protein stores vary with the amount of protein in the diet and that abundant stores make the animal more resistant to the debilitating effects of caloric restriction. As yet, adequate criteria are not available for the definition of satisfactory or optimum protein stores.

EFFECT OF POTASSIUM IODIDE ON LACTATION

Iodinated casein feeding has been rather widely used experimentally for the past ten years as a lactation stimulant in the bovine (*Nutrition Reviews* **11,** *333 (1953)*). The results have been quite variable, and the general use of iodinated casein has been questioned owing to the undesirable effects on the metabolic activity of the animal.

Workers at Cairo University in Egypt have recently reported that potassium iodide feeding resulted in lactation stimulation in the Egyptian buffalo (I. Sirry and H. A. Hassan, *Empire J. Exp. Agr.* **23,** *214 (1955)*). The authors state that this simple inorganic salt of iodine contains an unvarying quality and quantity of iodine and its use might therefore result in less variable influences on the metabolic activity of the animals.

Six pairs of buffaloes, each pair being as nearly identical as possible in age, calving time, stage of lactation and milk production, served as experimental animals. One member of each pair received the usual ration whereas the other member received the same ration and one tablet each day containing 120 mg. of potassium iodide. The experiment lasted from eleven to thirty-three

weeks depending upon the stage of lactation the animal was in initially.

A summary of the data, up to the time when one member of a pair ceased to lactate, indicated an over-all stimulation of lactation of 20 per cent from the potassium iodide feeding. The total milk production of all animals receiving the control ration was 3725 kg., contrasted with a total production of 4517 kg. in animals fed potassium iodide. The response to iodide between individual pairs of animals ranged from 10 to 34 per cent. In addition to the above response, 4 animals receiving iodide exhibited a prolongation of lactation over their respective controls that resulted in still further production of 841 kg. of milk by the iodide-fed animals.

There was a considerable lag in response to the iodide feeding. Animals exhibiting the greatest stimulation in lactation were those receiving the compound for at least twenty weeks.

Milk composition was also observed to be influenced by the iodide feeding. The acidity, specific gravity, nonfat solids, total nitrogen, casein nitrogen, and albumin, globulin, and proteose nitrogen of the milk of the iodide group were all higher than those of the control group. Only acidity, specific gravity and nonfat solids were significantly increased, however. The iodide feeding resulted in a significant lowering of the milk fat (from 7.58 per cent to 7.28 per cent). The influence of iodide on milk composition was in general similar to that observed when iodinated casein or thyroprotein are fed.

The undesirable side effects on metabolism that are often seen with thyroprotein feeding, such as accelerated respiration and pulse rates, and increase in body temperature, were not observed from potassium iodide feeding. Mean body temperature, determined twice daily, was identical between iodide-fed and control animals (38.3° C.). The mean pulse rate of 46 per minute was the same in the two groups of animals.

These results are of unusual interest, and it is surprising that the wide use of iodized salt in this country has not resulted in reports of similar stimulation of lactation in the bovine. The Egyptian workers did not report on the influence of the iodide supplement on the concentration of iodine in the milk. One would expect a considerable increase since the iodide ion is known to pass into the mammary tissue. The level used (120 mg. of potassium iodide daily), an intake of 92 mg. of iodine, exceeds by a considerable amount the 2 micrograms per kilogram of body weight per day that is currently considered adequate for the bovine.

THE LOCUS OF INSULIN ACTION

In the last ten years two major hypotheses have been postulated to explain the action of insulin. The first, the "hexokinase" hypothesis brought forward by the Cori laboratory (W. H. Price, C. F. Cori, and S. P. Colowick, *J. Biol. Chem.* **160,** *633 (1945)*), suggests that insulin influences the activity of hexokinase (glucokinase), the enzyme concerned with the conversion of glucose to glucose-6-phosphate. The second hypothesis, presented by R. Levine, M. S. Goldstein, B. Huddlestun, and S. P. Klein (*Am. J. Physiol.* **163,** *70 (1950)*), suggests that insulin exerts its effect by increasing cellular permeability to glucose. Experimental evidence for and against both hypotheses has been adduced by numerous investigators.

In general two types of dissent to the hexokinase hypothesis have arisen. One denies the existence of an inhibition of the hexokinase reaction in diabetic animals which can be reversed by insulin (*see for example* W. C. Stadie, *Physiol. Rev.* **34,**

52 (1954)). The other, while admitting an inhibition at the hexokinase level in the diabetic preparation, doubts the necessity of intact cellular structure for demonstration of this inhibition (*see* M. E. Krahl *in* "The Hypophyseal Growth Hormone, Nature and Actions," edited by R. W. Smith and others, *p. 409, McGraw-Hill Book Co., New York (1955)*).

The "cellular permeability" hypothesis was first tested by Levine and co-workers in nephrectomized-eviscerated dogs and rats. D-galactose was the sugar chosen to study the effect of insulin on sugar transfer into cells because of the absence of galactokinase from muscle. The volume distribution of galactose increased from 45 per cent of the body weight before insulin to 70 per cent of the body weight after insulin treatment. The distribution of other sugars such as D-arabinose and D-xylose, which are identical to D-glucose and D-galactose in carbons 1, 2 and 3, was similarly increased in volume distribution after insulin treatment (M. S. Goldstein, W. L. Henry, B. Huddlestun, and R. Levine, *Am. J. Physiol.* **173,** *207 (1953)*).

Recently A. Beloff-Chain and co-workers (*Proc. Roy. Soc. (London) B* **143,** *481 (1955)*) assayed the effects of insulin on carbohydrate metabolism in the isolated rat diaphragm by means of uniformly labeled glucose-C^{14}. Hemidiaphragms from normal and alloxan diabetic rats were incubated aerobically as well as anaerobically in both phosphate and bicarbonate mediums containing the glucose-C^{14}. Glucose metabolites were separated by paper chromatography and the radioactivity of the various metabolites was determined. The results of these experiments showed that there was no difference in glucose uptake or metabolism in diaphragm muscle from normal and diabetic rats. Insulin increased the glucose uptake in both normal and diabetic diaphragms. In normal diaphragms the percentage of glucose disappearing from the medium which was converted to carbon dioxide was actually decreased in the presence of insulin. The latter finding does not agree with that of C. A. Villee and A. B. Hastings (*J. Biol. Chem.* **179,** *673 (1949)*), who reported insulin increased carbon dioxide production in the diaphragm. Insulin appeared to affect primarily the conversion of glucose to glycogen and oligosaccharides and not to influence the reactions of oxidative degradation in the diaphragm muscle.

Beloff-Chain and her colleagues interpret the results of these experiments as opposed to either the "cellular permeability" or "hexokinase" hypotheses of the action of insulin. In regard to the "cellular permeability" postulate, these workers could find no effect of insulin on galactose disappearance in the absence of glucose. The increased galactose uptake after insulin administration reported by Levine *et al.* may therefore have been only a secondary insulin effect.

As regards the "hexokinase" hypothesis, Beloff-Chain and her co-workers could find no consistent stimulating effect of insulin on the hexose-monophosphate fraction. At the beginning of aerobic incubation when insulin exerted a marked effect on incorporation of glucose into glycogen, significant amounts of hexose-monophosphate could not be detected. Increasing amounts of this fraction accumulated with increasing incubation time. Furthermore, under anaerobic conditions most of the glucose disappearing from the medium accumulated as free glucose in the diaphragm muscle cells.

Beloff-Chain *et al.* conclude that insulin may be primarily concerned with the reactions involved with the polymerization of glucose to glycogen and that the accelerating effect on these reactions which insulin produces cannot be satisfactorily explained on the basis of present knowledge of the mechanism of glycogen synthesis. The complete inhibition of glycogen synthesis from glucose in rat diaphragm produced by dinitrophenol (A. Beloff-Chain *et al., Atti accad. nazl. d. Lincei, Rend. Classe sci. fis. mat. e nat.* [8], **14,** *749 (1953)*) and by anaerobic incubation makes it plausible

that glycogen synthesis is coupled with some aerobic phosphorylation process. It is further speculated that in this process some unstable phosphorylated intermediate ("active glucose") might be found.

The work of Beloff-Chain and co-workers is of interest in pointing up possible areas where research on the locus of action of insulin might be directed. However, it has the failings of all *in vitro* work which is applied to *in vivo* systems. Certainly in the diabetic animal there is a failure in the peripheral uptake of glucose in the large bulk of the muscle mass which was not found in the *in vitro* experiments reported here. Certainly, also, one would hesitate to abandon either the "hexokinase" or "cellular permeability" hypotheses on the basis of the evidence reported by Beloff-Chain *et al.*

MINERALS AND TOOTH DECAY

In an earlier review the influence of the ash of laboratory chow in reducing caries incidence, when fed in place of a reagent-grade salt mixture during tooth development, has been discussed (*Nutrition Reviews* **13,** *29 (1955)*). A. E. Nizel and R. S. Harris (*J. Dent. Res.* **34,** *513 (1955)*) have now discussed an experiment in which they included the ash of a natural diet in the ration of hamsters.

Two groups of these animals were used. One was fed diet A which was composed of ground whole corn, whole milk powder, alfalfa meal and sodium chloride. Some of this diet was ashed in a muffle furnace at 510° C. The second group was fed diet B, which was diet A supplemented with 3 per cent of this mineral ash. The total ash contents of diets A and B were 3.46 and 6.42 per cent, respectively.

These diets were fed to female hamsters beginning at the time their young were born. The offspring were weaned at 21 days of age, placed in individual cages and continued on the same diet as their mothers until they were 20 weeks of age. Altogether, 24 young hamsters were raised by females fed diet A, and 17 by mothers that had been fed diet B.

At the end of the experiment, of the 17 hamsters which had received ash-supplemented diet B, 13 had no carious lesions and 4 had one small lesion each. Of the 24 hamsters which had received the diet without additional ash, 4 had no carious lesions and the remaining 20 had caries scores ranging between 0.05 and 0.95 with an average of 0.18. These results were interpreted to mean that additional ash from these foodstuffs exerted a caries-inhibiting effect when fed to female hamsters at the time their young were born and to the young thereafter.

The critical reader of this manuscript should have numerous reservations about this conclusion. For example, on the surface it would appear that a definite influence of the ash had been observed; however, the low incidence of tooth decay in this experiment makes interpretation of the data rather hazardous in view of the small number of female hamsters that must have been fed either diet A or diet B and the unknown heredity and dietary background of the females. The authors do not state how many pregnant females were in each group, although with the number of offspring involved one would expect 4 or 5 females per group. Neither do they state whether there was any blood relationship among the females. With the wide differences in caries-susceptibility that have been shown in every species of rodent used for experimental caries studies, every investigator should lean over backward to give his experiment the benefit of hereditary control. This is especially true in an experiment where a second generation is involved, with the additional uncontrollable

factors that are thus brought into the experiment.

If the data in this study are of significance it would appear that at least part of the caries-inhibiting effect was due to a systemic influence, since the diet change began prior to the development of the teeth. The experiment would have been more valuable if larger groups had been used so that each litter could have been divided at weaning time into two groups per litter. One group could have continued on the mother's diet while the other half of each litter could have been changed over to the other diet. In this way additional information about the possible postdevelopment versus systemic influence of the ash supplement could have been realized.

P. H. Keyes (*J. Dent. Res.* **35,** *95 (1956)*) has also studied the influence of the ash residues of certain natural diets and natural foodstuffs on the dental caries index among Syrian hamsters, under more carefully controlled circumstances than the previously described experiment. In his studies he tested the influence on tooth development of a diet that contained mainly purified materials with the minerals supplied by whole milk powder at a 5 per cent level and from the ash of laboratory chow at a 4 per cent level. Another group was fed substantially the same diet except that in place of the ash from laboratory chow, an ash prepared from whole wheat flour was substituted. In the latter case, the 5 per cent level of whole milk powder was still maintained, and the whole wheat ash was fed at a 2 per cent level. In still another group of animals the powdered milk was removed from the diet and replaced percentagewise by sucrose; the entire mineral content of the diet was supplied by whole wheat ash at a 4 per cent level. A fourth group was fed the latter diet supplemented with 1 per cent calcium hydroxide. Under these circumstances, offspring of female hamsters that were maintained on the two diets containing the whole wheat ash had high levels of caries-susceptibility, while those that were fed the diet containing the ash of laboratory chow or the diet supplemented with a 1 per cent level of calcium hydroxide had a much lower caries-susceptibility. The hamster females in these studies were closely related and in a number of instances were restudied with different diets during successive litters to provide a close genetic control.

Whereas the above studies were conducted under circumstances that partially implicate the development and calcification of the teeth, numerous other studies have been conducted after the teeth were fully developed to study the influence of certain elements on the caries process itself independently of tooth development. For example, J. Wisotzky and J. W. Hein (*J. Dent. Res.* **34,** *735 (1955)*) have conducted a series of studies to determine the relationship of the caries-inhibiting potentiality of cations to their position in the electromotive series. In general, their working hypothesis was that cations above hydrogen in the electromotive series would not inhibit experimental dental caries, while oxidizing cations below hydrogen would inhibit caries. In the course of these tests, magnesium, lithium, cadmium and lead were chosen as four representative cations with positions above hydrogen. These elements were provided to hamsters, after tooth development was largely completed, in the drinking water at a level of 0.5 milliequivalents (mEq) of the compound per liter of water, except in the case of cadmium where it was found that this level had to be reduced by half in order to prevent acute toxicity.

The results of these studies indicate that this concentration of lithium as lithium sulfate had relatively little effect on the dental caries incidence, with a 10 per cent increase in males and a 17 per cent decrease in females. The same concentration of magnesium as magnesium sulfate caused a 39 per cent increase in dental caries among

male hamsters and a 53 per cent increase among females. The cadmium as cadmium sulfate caused an 11 per cent increase in tooth decay among males and a 99 per cent increase among females. The lead supplement as lead acetate produced a 38 per cent increase in males and a 56 per cent increase among females. In a later series of experiments (J. Wisotzky and J. W. Hein, *Proc. Internat. Assn. Dent. Res., 34th General Meeting (1956)*) the same investigators reported the influence of platinum, palladium and gold additions to the water supply on the incidence of tooth decay in male hamsters. These three cations were chosen as representative of the area below hydrogen in the electromotive series. The supplement of platinic chloride caused an increase in dental caries in the neighborhood of 87 per cent. The palladium supplement had virtually no effect on the caries score and the gold chloride solution had a strong inhibiting effect upon the caries rate, producing a reduction in the neighborhood of 80 per cent. Obviously these findings do not fit the original postulate concerning the oxidative ability of the cations below the position of hydrogen in the electromotive series.

In another experiment these workers (J. W. Hein and J. Wisotzky, *J. Dent. Res.* **34,** *756 (1955)*) studied the influence of a 10 parts per million (p.p.m.) solution of vanadium (vanadium pentoxide dissolved in hydrochloric acid and adjusted to pH 6.5) on dental caries in male and female Syrian hamsters. They observed an increase of 35 per cent in the total caries score among males and a 33 per cent increase in the total caries score among females. These results are in direct contradiction to those of C. F. Geyer (*Ibid.* **32,** *590 (1953)*). However, Hein and Wisotzky point out that the wide range of valencies in vanadium ions and the numerous acid, hydroxide and oxide combinations might mean that the ion actually ingested by their rats was different from those discussed by Geyer. In another experiment, Hein and Wisotzky (*loc. cit.*) tested the influence of a solution containing 1 mEq of potassium permanganate on the incidence of dental caries in male and female hamsters after tooth development was largely completed. The experimental males had an increase of 9 per cent in total caries score over the controls, while the experimental females showed a 29 per cent decrease in score. Neither of these changes was statistically significant.

These studies can be divided into two general categories on the basis of the period in the life history of the subjects when the experiments were begun. In one category are all of the trials by Wisotzky and Hein which were begun after weaning when tooth development is largely completed except for the third molar. Hence these studies concern the influence of the various ions tested on the finished tooth through an oral environmental circumstance or through some presently undefinable systemic influence. This is a potential means to explore the caries process in the hope of obtaining a better understanding of its initiation and progression. Obviously, from the data presented by Wisotzky and Hein, their working hypothesis of a relationship between the position of the cations in the electromotive series and their caries-inhibiting and caries-enhancing abilities did not stand experimental test. At the present time, no workable hypothesis appears to exist for the explanation of the divergent results from the several cations tested.

In the other category are the studies by Nizel and Harris and by Keyes which were begun either before the beginning of tooth development or in its early stages. Thus the experimental manipulations in these studies have the potential ability to influence the formation of teeth in ways that may alter their basic susceptibility to tooth decay rather than or in addition to influences of a postdevelopmental nature on the caries process itself. These potential developmental influences have received much less attention than the postdevelopmental ef-

fects. In other words, the emphasis has been placed on changing the oral environment in such a way as to modify the initiation and progression of carious lesions in teeth of predetermined caries-susceptibility. Unquestionably this is an important phase that should not be overlooked. However, there is greater need for more excursions into the more arduous and complicated area of tooth development in the hope of being able to modify the tooth's composition and structure in ways that will increase the predetermined caries-resistance. Obviously there are many facets to be controlled rigorously in such developmental studies. Failure to exert such control will result in questionable data that may becloud the field and inhibit progress.

NUTRITIONAL FACTORS IN LIPOTROPISM BY PROTEIN

The lipotropic action of protein has been variously ascribed to its methionine content (*Nutrition Reviews* **10,** *187* (*1952*)), its content of certain other amino acids such as threonine and lysine (*Ibid.* **12,** *22*, *151* (*1954*)) and its properties as intact protein (*Ibid.* **13,** *217* (*1955*)). An apparent disagreement in the reported results of two groups of investigators was resolved when S. A. Singal, S. J. Hazan, V. P. Sydenstricker, and J. M. Littlejohn (*J. Biol. Chem.* **200,** *867* (*1953*)) found that threonine has lipotropic activity only in the presence of some dietary choline. Thus the report of H. C. Eckstein (*Ibid.* **195,** *167* (*1952*)) that the lipotropic activity of protein resides solely in its methionine content can be explained by the absence of choline from the diets used in their experiments. The resolution of this discrepancy, however, was accompanied by the realization that dietary factors other than those under immediate investigation may have a profound influence on the results.

Recently, A. E. Harper and D. A. Benton (*Biochem. J.* **62,** *440* (*1956*); *see also Nutrition Reviews* **14,** *225* (*1956*)) investigated a number of factors influencing the lipotropic action of protein. Using groups of 6 young rats each, these authors first confirmed previous information that when 9 per cent casein of the basal diet was supplemented with 6 or 12 per cent gelatin, liver fat was reduced except in experiments in which the 0.15 per cent choline of the basal diet was replaced by 0.7 per cent extra methionine, for a total of 1 per cent. Although in the latter case the gelatin induced an increased growth rate, it was shown that the fatty liver was not caused by a resultant increased use of methionine, since further increases in the percentage of dietary methionine to the point of toxicity did not decrease the liver fat. Moreover, exclusion of supplementary tryptophan (0.1 per cent) from the diet did not decrease liver fat although growth was inhibited to the same degree in both cases.

When the gelatin was replaced by its content of glycine and arginine, no decrease in liver fat resulted. A supplement of 0.36 per cent of DL-threonine had some lipotropic action by itself, but this was reversed by arginine, glycine and especially guanidino acetic acid. Since these latter compounds are known to be methylated by methionine, it is probable that their action may be explained as a competition for the available methionine, which could not be increased much beyond the 1 per cent level because of toxic effects.

When the casein content of the diet was raised to 20 to 40 per cent none of the amino acid supplements or gelatin had any further effect. With the lower amount of casein, betaine hydrochloride had better lipotropic activity than excess methionine, glycine, or ethanolamine, although all were markedly inferior to choline.

In all these experiments, young rats were used and kept on the experimental diets

less than four weeks. If animals on an 18 per cent casein diet or 9 per cent casein supplemented by 12 per cent gelatin, and supplemented, in both cases, by 0.7 per cent methionine, were allowed to continue on the diets for more than four weeks, the high liver fat gradually decreased to normal values, which in the case of the 18 per cent casein diet were as low as those obtained with the choline supplement. Apparently, in the presence of adequate protein, the young rat can adapt to the efficient use of methionine for choline synthesis.

Harper and Benton explain these findings as the result of reduced choline synthesis in those cases in which fatty livers are obtained. In the low casein experiments, the need for adequate dietary protein was evidently not satisfied, as it was with the 18 per cent casein, supplemental gelatin, or threonine. The glycine, arginine and guanidino acetic acid probably acted by decreasing the methionine stores available for choline synthesis.

Three distinct functions of the nonsulfur-containing amino acids may be deduced from these experiments. First, adequate protein is necessary in young rats for synthesis of choline from methionine (threonine and other essential amino acids may function in this manner). In this case, unless lipotropic agents such as choline are present in adequate amounts, the function of the protein is to increase growth and food consumption with consequent production of fatty livers. Second, specific amino acids such as threonine, lysine and tryptophan may have specific effects unrelated to adequacy of the protein for growth. Finally, protein itself may play an important role in lipid transport.

Although these experiments have thrown some light on a poorly understood and complex subject, much work remains to be done. The complexity is perhaps best illustrated by the multiplicity of factors which may lead to production of fatty livers. Choline has been implicated both in the transport of fat from the liver (as lecithin) and as necessary for fatty acid oxidation in the liver (C. Artom, *J. Biol. Chem.* **205,** *101 (1953)*). Methionine functions in choline synthesis while threonine may owe its activity both to this function and to some more specific action in fat oxidation (S. A. Singal, S. J. Hazan, V. P. Sydenstricker, and J. M. Littlejohn, *Ibid.* **200,** *875 (1953)*). Finally, the mechanism of transport of fat from the liver and the role of lipoproteins as agents for such transport should be investigated.

EFFECT OF CORTISONE ON PROTEIN METABOLISM

The classic paper of C. N. H. Long, B. Katzin, and E. G. Fry (*Endocrinology* **26,** *309 (1940)*) described the role of the adrenal cortex in promoting gluconeogenesis from proteins during fasting. The mechanism of this effect has been under investigation by a number of workers since the appearance of this paper. The application of the isotope of nitrogen, N^{15}, to the study of protein metabolism was made by D. Shemin and D. Rittenberg (*J. Biol. Chem.* **153,** *401 (1944)*), and has provided a powerful tool for unraveling the complexities of nitrogen metabolism. H. D. Hoberman (*Yale J. Biol. Med.* **22,** *341 (1950)*) analyzed the metabolism of N^{15}-glycine in rats in the presence or absence of cortisone. The results were interpreted as indicating an increased rate of protein catabolism. Other workers using different experimental approaches have attributed the action of corticosteroids to an inhibition of protein synthesis.

A new approach to this difficult problem has been made by I. Fritz (*Endocrinology* **58,** *484 (1956)*), who wished to differentiate between the effects of adrenal cortical steroids upon incorporation of labeled

amino acids into tissue proteins, and their possible effects on the release of label which had been incorporated into tissue proteins prior to establishment of an endocrine imbalance. Glycine-N^{15} was administered orally to rats over a three-day period. One group of the glycine-treated animals was then adrenalectomized, a second group received 5 mg. per day of cortisone and the third was the control group. The animals were then starved for periods up to 161 hours.

As was predicted, the cortisone-treated animals excreted the greatest amount of urinary nitrogen during the starvation period. The control group excreted intermediate amounts of nitrogen and the adrenalectomized rats excreted the least nitrogen. A comparison of the distribution of body proteins during starvation by normal and cortisone-treated rats is of interest. A significantly smaller fall in liver protein nitrogen occurred in the cortisone-treated group than in normal rats. There was also a greater loss of protein content in the thymus and spleen of the cortisone-treated rats. It is well established that administration of adrenal steroids leads to the lysis of lymphoid tissue, and the loss of protein from these lymphoid organs can be attributed to this action.

The percentage of administered N^{15} which was excreted during the seven-day period of starvation was greatest for the cortisone-treated group, least for the adrenalectomized group, and intermediate for the normal animals. Of considerable interest was the observation that the actual degree of enrichment of urinary nitrogen with N^{15} was inversely related to the total nitrogen excretion. This was interpreted as indicating that there is a mechanism by which recently "synthesized" protein is catabolized by the organism preferentially. Examination of the N^{15} content of tissue proteins from normal and cortisone-treated rats failed to demonstrate large differences between the organs of cortisone-treated and control rats, with the exception of a greater fall in thymus N^{15} content in the cortisone-treated group. Of those tissues with a high initial activity of N^{15}, liver, kidney and plasma proteins exhibited the most rapid loss of N^{15}. It was concluded that net shifts in total protein nitrogen among selected tissues and organs were markedly influenced by adrenalectomy and cortisone treatment. In these experiments the most striking observation was the increased liver protein content in cortisone-treated fasting rats. Caloric deficiency alone is not responsible for this finding, because R. H. Silber and C. C. Porter (*Endocrinology* **52,** *518 (1953)*) have noted that cortisone will protect the liver proteins from depletion during feeding with low protein diets.

L. A. Pesch and J. H. Clark (*Proc. Soc. Exp. Biol. Med.* **91,** *510 (1956)*) have carried out *in vitro* studies in an attempt to explain the paradoxic action of cortisone on liver proteins in protein deficiency. The uptake of glycine-2 C^{14} by isolated diaphragms and liver slices of protein-deficient rats with and without cortisone has been compared. Dietary deficiency of protein actually was found to stimulate the ability of diaphragm to incorporate the labeled amino acid. The incorporation was increased further by the administration of cortisone to the protein deficient animals. Liver slices from rats fed a low protein diet had a decreased ability to incorporate glycine into protein as compared to slices from rats on a normal dietary protein intake. The synthetic rate was stimulated by the administration of cortisone. From these studies the authors concluded that cortisone does not act simply by depressing protein synthesis. The action of cortisone was believed to be organ-specific in promoting greater catabolism of protein in certain organs such as diaphragm than in liver.

These new contributions to our knowledge of the interrelation of cortisone and protein metabolism are of interest but do not permit as yet an explanation of the many conflicting

observations in this field. In defining the action of cortisone it is necessary to consider the dietary conditions of protein and caloric intake, because certain actions of cortisone can only be made evident in the presence of dietary deficiency. This has important clinical implications. The therapeutic use of cortisone in patients ingesting an adequate diet has not been associated with major changes in nitrogen balance unless excessive doses of the hormone have been given. Under many circumstances positive nitrogen balance has actually been observed. When, however, protein and caloric intakes are inadequate, the net synthesis of protein by the body may be greatly impaired by cortisone administration. In applying the experimental observations to clinical problems the question of steroid dosage arises. In general the experimental studies are carried out with amounts of cortisone which are grossly excessive and tend to exaggerate certain processes which might not be operative at levels of the steroid used in replacement therapy or in the lower levels of steroid therapy used for therapeutic purposes.

Another important concept which is emphasized by both of these studies is that generalizations on the action of hormones on the basis of over-all nitrogen balance may lead to false simplifications. It is apparent that various organs and tissues react to cortisone to different degrees and sometimes in opposing directions. The multiplicity and complexity of the nitrogen pools of the body thus make the analysis of hormonal effects difficult.

ENZYME CHANGES IN COPPER DEFICIENCY

Copper deficiency has been shown to result in a microcytic hypochromic anemia in several species of animals. The similarity of this anemia to that resulting from iron deficiency is obvious and there are considerable data which suggest that copper is required for normal iron metabolism. In copper-deficient animals there is an impairment in the absorption of iron, in the mobilization of tissue iron, and in the utilization of parenterally administered iron (*Nutrition Reviews* **11,** *336* (*1953*)).

One of the specific effects of copper on iron metabolism appears to rest in the synthesis of the heme ring (iron-porphyrin) which is the prosthetic group of hemoglobin, cytochromes and other important biochemical compounds. The dependence of cytochrome *a* on normal copper nutrition in yeast was demonstrated by C. A. Elvehjem (*J. Biol. Chem.* **90,** *111* (*1931*)) and in animal tissue by E. Cohen and Elvehjem (*Ibid.* **107,** *97* (*1934*)). Particularly striking were the results of M. O. Schultze (*Ibid.* **138,** *219* (*1941*)), in which the cytochrome oxidase (a cytochrome *a* component) activity of bone marrow was shown to depend on the dietary copper level.

However, all the signs of copper deficiency in animals may not be related to a defect in iron metabolism. Copper-deficient pigs exhibit an abnormal leg condition which resembles rickets, and atrophy of the leg muscles (*Nutrition Reviews* **9,** *317* (*1951*)). In sheep, copper deficiency results in neurologic disturbances in the offspring and in deterioration of the fleece (*Ibid.* **8,** *280* (*1950*)). Such findings, coupled with the possibility that earlier experiments on the relation of copper to enzyme activities were complicated by other deficiencies, have led C. H. Gallagher, J. D. Judah, and K. R. Rees (*Proc. Roy. Soc.* (*London*) *B* **145,** *134* (*1956*)) to a comprehensive study of enzyme activities in copper-deficient rats.

Weanling rats were given a basal diet of hydrogen sulfide-treated milk supplemented with iron and other minerals, and with vitamins. Control animals received in addition 50 micrograms of copper daily.

Animals receiving the copper-deficient diet exhibited a reduced growth rate after approximately three weeks of feeding, anemia appeared after about ten weeks, and the animals began to die after three and one-half months. Animals in various stages of copper deficiency and their controls were killed and tissues taken for enzyme determinations, although some experiments required liver biopsy samples. A total of fourteen different enzymes or enzyme systems was assayed, including choline oxidase, transmethylase, catalase, cytochrome oxidase, and several enzymes involved in the Krebs tricarboxylic acid cycle.

Of the enzymes studied, only cytochrome oxidase (the terminal enzyme of biologic oxidation) showed a marked decline in copper deficiency. The succinic oxidase activity was also decreased, but this was apparently secondary to the cytochrome oxidase involved in this enzyme system. After only one month on the copper-deficient diet, the cytochrome oxidase of the liver had decreased to one fourth of its normal value, a stage at which the copper content of the liver had decreased to about one-half the normal value. In more severe copper deficiency, the cytochrome oxidase activity of liver approached zero. There were less marked decreases in the oxidase activities of kidney and brain. It was of interest that addition of copper salts to the oxidase assay medium or preincubation of the liver homogenate with copper did not restore the cytochrome oxidase level, while feeding of copper for eight to thirteen days restored the liver cytochrome oxidase activity nearly to normal.

The hemes were extracted from liver mitochondria prepared from copper-deficient and control animals and measured spectrophotometrically. Heme *a* (derived from cytochrome oxidase) was nearly completely lacking in the copper-deficient animals. Thus it seems evident that copper has a role in the synthesis of the iron-containing heme prosthetic group of cytochrome oxidase.

The authors do not feel that copper has simply a general effect on iron metabolism since certain other iron-containing enzymes were not depressed in copper deficiency. Thus DPN-cytochrome *c* reductase, a flavoprotein which contains iron but not in heme form, was not decreased in activity. Perhaps more pertinent was the failure to find any decrease in the activity of catalase, an enzyme which contains the same protohemin prosthetic group as hemoglobin. It is not unprecedented, however, that one metabolic process may be more critically dependent than another on a given nutrient or catalyst. In the case of copper deficiency it would appear that synthesis of the heme prosthetic group of cytochrome oxidase is influenced early, that of hemoglobin somewhat later, and that of catalase is least sensitive. The exact mechanism of the action of copper in the synthesis of the iron-porphyrin ring is completely unknown.

VITAMIN B_{12} AND LIVER ENZYMES

It is known that vitamin B_{12} is in some manner associated with nucleic acid metabolism (*Nutrition Reviews* **9,** *185 (1951)*; **12,** *293 (1954)*), with the metabolism of sulfhydryl groups (*Ibid.* **11,** *304 (1953)*; J. W. Dubnoff and E. Bartron, *Arch. Biochem. Biophys.* **61,** *99 (1956)*; *Nutrition Reviews* **14,** *244 (1956)*), and with the synthesis of methyl groups (*Ibid.* **8,** *269, 301 (1950)*; **11,** *220 (1953)*; **12,** *218 (1954)*; W. T. Wong and B. S. Schweigert, *Arch. Biochem. Biophys.* **60,** *126 (1956)*). However, insufficient information is available to pinpoint the exact metabolic role of this vitamin.

Several studies have been made on the effect of vitamin B_{12} on the concentration

of liver enzymes. M. B. Gillis and R. J. Young (*Poultry Sci.* **30,** *468 (1951)*) found no effect of vitamin B_{12} deficiency on liver choline oxidase, although they do not state the severity of the deficiency. J. N. Williams *et al.* (*J. Biol. Chem.* **202,** *151 (1953)*) and Williams, W. J. Monson, A. E. Harper, and C. A. Elvehjem (*Ibid.* **202,** *607 (1953)*) reported that a vitamin B_{12} deficiency in growing rats resulted in a marked decrease in liver endogenous respiration, in xanthine oxidase, and in betaine-homocysteine transmethylase activity. The lowering of xanthine oxidase activity was found to be indirect and associated with changes in general protein metabolism. A slight increase was seen in choline oxidase activity due, it was thought, to the increased endogenous respiration. S. P. Mistry *et al.* (*J. Biol. Chem.* **212,** *713 (1955)*) reported that vitamin B_{12} appeared to have no direct effect on the transmethylase content of the livers of the chick, rat and pig.

Of interest is the recent finding that in vitamin B_{12}-deficient rats there is a lowering of liver cytochrome oxidase, an iron-porphyrin-containing enzyme of basic importance in cellular biochemistry (B. L. O'Dell, J. S. Gordon, J. H. Bruemmer, and A. G. Hogan, *J. Biol. Chem.* **217,** *625 (1955)*). Tissues from newborn rats (24 hours old), offspring of vitamin B_{12}-deficient dams, were used. The dams had been depleted of vitamin B_{12} by maintaining them from weaning on a soybean meal diet deficient in vitamin B_{12}. The control animals received the same diet supplemented with 30 micrograms of vitamin B_{12} per kilogram of diet. A high percentage of the vitamin B_{12}-deficient offspring had hydrocephalus or congenital defects and usually died in three days with spasms, uremia and cyanosis (*see also* M. O. Schultze, *Proc. Soc. Exp. Biol. Med.* **72,** *613 (1949)*). Analysis of the livers of 5 deficient animals showed an average vitamin B_{12} activity of 13 millimicrograms per gram as compared with 346 millimicrograms per gram for the control animals, demonstrating the severity of the deficiency.

It was found that the vitamin B_{12} deficiency resulted in about a 50 per cent decrease in the level of liver cytochrome oxidase below that of the control animals. This was a highly significant change. The difference could not be accounted for by the small changes found in the dry matter or in the nitrogen content of the livers of the two groups. Furthermore, as shown by "paired fasting" experiments, the differences were not explained by starvation of the deficient newborn rat. In contrast to the effect in liver, the cytochrome oxidase content of other tissues (brain, kidney and skeletal muscle) from deficient animals remained normal. Additional studies made with liver cytochrome oxidase in 17 8 to 10 week old vitamin B_{12}-deficient rats from partially depleted dams confirmed the findings obtained with the newborn rats.

Studies on other liver enzymes were made on the newborn rat deficient in vitamin B_{12}. However, no significant differences could be found in endogenous respiration or in choline oxidase and succinoxidase activity. The differences between these results and those of Williams and co-workers (*loc. cit.*) may be due to differences in experimental technics, including the method of obtaining vitamin B_{12}-deficient rats and the age of the animal tested.

The results suggested to O'Dell and co-workers that vitamin B_{12} may have a specific function in the synthesis of the porphyrin-containing proteins of the body by some as yet unexplained mechanism. Unpublished work was cited indicating that vitamin B_{12} did not function as a coenzyme for cytochrome oxidase nor did the deficiency of vitamin B_{12} produce an inhibitor. Whatever the exact mechanism of action of vitamin B_{12} is in maintaining a normal level of liver cytochrome oxidase, these studies provide another clue for the much sought metabolic role of vitamin B_{12} in cellular nutrition.

FAT DEPOSITION IN CORONARY ARTERIES OF RATS EXPOSED TO COLD

Few well controlled studies of the physiologic and pathologic effects of prolonged exposure of the experimental animal to a cold environment have been recorded. The recent observations of E. A. Sellers and R. W. You (*Brit. Med. J.* **1,** *815 (1956)*) indicate that this may be a fruitful field for study. These investigators have recorded a high incidence of lipid-containing coronary lesions in rats chronically exposed to temperatures of 1 to 3°C. for periods of several months.

The first experiment was of particular interest since the experimental and control groups of Wistar rats were both fed a stock ration, the only notable variable being that the experimental group was kept in a cold room for ten to eighteen months while the control animals were maintained at 22°C. Eighteen of 29 experimental animals showed stainable lipid in their coronary arteries while none of the 27 control rats demonstrated similar deposits. A few of the rats exposed to cold also demonstrated small lipid-containing lesions in their aortas. Although Sellers and You found no significant hypertension in the cold-treated rats, they did note a definite increase in kidney and heart weights in this group. Furthermore, there was microscopic evidence of more renal injury in the animals exposed to the cold. This consisted of hyaline changes in the glomerular loops, fatty deposits in the glomeruli and degenerative changes in the tubule cells with associated cast obstruction. Arteritis of the type frequently observed in old rats was seen in both the control and experimental animals.

In a second experiment Sellers and You (*loc. cit.*) demonstrated that twelve to thirteen months exposure to cold resulted in a moderate increase in total blood lipids from 425 to 675 mg. per cent. Similarly the blood cholesterol, both free and bound, rose from 44 and 15 mg. per cent respectively to 69 and 22 mg. per cent. Less elevation in serum cholesterol was observed after nine months and little or no elevation at one to five months in the cold.

In the third experiment the role of choline in the pathogenesis of these cold-induced lesions was studied. In this experiment four groups of 15 rats weighing about 150 g. initially were studied over a six-week period. They were fed a hypolipotropic ration containing 34 per cent lard and 2 per cent cholesterol with the protein furnished by peanut meal, soya protein and casein. Two groups receiving this ration, one with choline supplementation and one without, were kept at 1 to 3°C. and two similar groups were maintained at 22°C. In this relatively brief experimental period several noteworthy observations were made. Many of the choline-deficient rats failed to survive the six-week experiment, those at room temperature apparently succumbing to the severe kidney damage of choline deficiency while those at 1 to 3°C., showing much less kidney damage and less lipid in their livers, apparently died of other causes. Of the two groups of rats maintained at room temperature, only the group with choline deficiency showed widespread fatty lesions in the coronary arteries. Paradoxically, the rats housed in the cold showed a high incidence of fat deposition in their coronary arteries only when they were fed the ration supplemented with choline. Although the choline-deficient rats kept in the cold showed widespread myocardial fatty degeneration and necrosis, no coronary lipid deposits were observed. While the kidneys of the "cold" caged, choline-supplemented group were enlarged, there was no microscopic evidence of renal damage which might have been interpreted as contributing to the coronary vascular disease. This group also showed the largest hearts, the largest adrenals and the least fat in the liver. Their food intake was the greatest, but they

gained less weight than the group fed the same diet while being kept at 22°C.

The results of this study raise many challenging questions which will undoubtedly engage the interest of students of the atherosclerosis problem. Geographic studies have recently emphasized the dietary differences which apparently correlate so well with the incidence of this disease in different parts of the world. These may have to be broadened to include a consideration of environmental temperature in the epidemiology of atherosclerosis. Obviously arterial injury of unknown etiology, but perhaps related to adrenal hyperfunction and renal injury, will have to be studied further as a factor predisposing to the lipid deposition in cold-engendered coronary lesions. Among the other endocrine factors to be evaluated in pursuing this stimulating study, the relationship of these lesions to the sex of the animal deserves special consideration. Although Sellers and You mention that animals of both sexes were studied, no correlation of incidence with the sex of the animal was included in their paper.

The results with the choline-deficient diets serve to extend the work of W. S. Hartroft *et al.* (*see Nutrition Reviews* **13,** *189 (1955)*; **14,** *193 (1956)*) and G. F. Wilgram, Hartroft, and C. H. Best (*Brit. Med. J.* **2,** *1 (1954)*; *Science* **119,** *842 (1954)*), who have demonstrated a correlation of this deficiency with coronary artery disease in the rat and emphasized its association with severe renal injury. The study also confirms the special susceptibility of the coronary arteries of the rat to lipid deposition, a characteristic shared by some human subjects. It further illustrates the usefulness of the rat in the study of experimental coronary atherosclerosis (*Nutrition Reviews* **13,** *20 (1955)*).

PLANT STEROL ABSORPTION IN THE RAT

Current interest in the relationship between dietary sterols and blood sterols is evidenced by the many reports that are available on this subject (*Nutrition Reviews* **14,** *39, 67, 107 (1956)*). Although the rat is quite resistant to atheromatosis, which in turn is often correlated with elevated blood cholesterol, several recent studies with rats are worthy of mention.

Studies made at George Washington University, Washington, D. C., and the Veterans Administration Center, Martinsburg, West Virginia, indicate that soybean sterols, added to a high cholesterol diet, inhibit the development of hypercholesterolemia in rats. *In vitro* studies showed that soybean sterols were esterified by pancreatic cholesterol esterase under the same conditions as cholesterol, namely, in the presence of bile salts and a fatty acid source (L. Swell, T. A. Boiter, H. Field, and C. R. Treadwell, *Proc. Soc. Exp. Biol. Med.* **86,** *295 (1954)*). Sterol specificity studies indicated that the same enzyme synthesized and hydrolyzed long-chain fatty acid esters of a number of sterols (beta-sitosterol, mixed sitosterols, dihydrocholesterol, ergosterol and stigmasterol) (Swell, Field, and Treadwell, *Ibid.* **87,** *216 (1954)*). It was postulated that plant sterols inhibit the absorption of cholesterol by competing with cholesterol for the enzyme and its cofactors. In view of the finding that the same enzyme catalyzed the esterification of both cholesterol and plant sterols, it seemed worthwhile to consider that plant sterols might be absorbed through the same mechanism as cholesterol.

The belief that plant sterols are not absorbed from the intestine is based on the classic studies of R. Schoenheimer (*Science* **74,** *579 (1931)*), who failed to increase the body sterols of rabbits and cats by feeding large amounts of plant sterols. He was further able to recover all plant sterols fed, in the feces, and was unable to detect any

sitosterol in the circulation. This earlier finding has been challenged by the more recent observation that tritium-labeled plant sterols are absorbed, although not so well as cholesterol (R. G. Gould, *Circulation* **10,** *589 (1954)*).

If the blood cholesterol-lowering effect of dietary plant sterols (*Nutrition Reviews* **14,** *107 (1956)*) is due to competition between these plant sterols and cholesterol (dietary and endogenous) for the cholesterol esterase enzyme system, then a maximum lowering should occur under cholesterol-free dietary conditions. This hypothesis was recently tested by L. Swell, T. A. Boiter, H. Field, and C. R. Treadwell (*J. Nutrition* **58,** *385 (1956)*).

Rats weighing 150 g. were divided into nine groups of 7 each. A basal synthetic lipid-low, cholesterol-low diet served as a control diet. This diet supplemented with 5 or 25 per cent of oleic acid, 2 per cent of soybean sterols, 1 per cent of sodium taurocholate, or combinations of these, served as the experimental treatment. The basal diet consisted of the following in per cent: casein, 20; corn starch, 24; glucose, 24; roughage, 2; salts, 5; and adequate amounts of the crystalline vitamins. The remainder of the diet consisted either of the supplement or additional carbohydrate.

Sterol excretion data (average of two seven-day collection periods) indicated no absorption when the basal diet was supplemented with 2 per cent of soybean sterols only. The same diet with 1 per cent of bile salts added (taurocholate) resulted in sterol absorption equal to about 16 per cent of the amount consumed. A similar absorption rate was apparent when the diet contained oleic acid. A combination of bile salts and oleic acid resulted in maximum plant sterol absorption (23 per cent).

Liver sterols were also analyzed. Oleic acid supplementation of the basal diet increased liver sterols about 25 per cent. Supplementation with bile salts resulted in a 75 per cent rise in liver sterols. A combination of oleic acid and bile salts resulted in a 114 per cent rise. Surprisingly, corresponding groups receiving soybean sterols all exhibited a smaller rise in liver sterols.

Paper chromatography was employed in an attempt to detect plant sterols in either the serum or livers of animals that had exhibited evidence of plant sterol absorption from fecal- and feed-balance data. No plant sterols were detectable in liver. In the group that had exhibited maximum plant sterol absorption, less than 5 per cent of the sterols in serum was detectable as sterols of the type fed. This was considered presumptive evidence of rapid destruction or conversion to sterols indistinguishable from cholesterol by the chromatographic technic employed.

The failure of plant sterol feeding to lower total circulating sterols in the rat, as has been reported to occur in human beings, should not be considered a conflicting result since in man no attempt was made to determine the influence of plant sterols under a cholesterol-low dietary condition.

NOTES

The Measurement of Skinfold Thickness

In a brief report (*Am. J. Clin. Nutrition* **4,** *35* (*1956*)) D. A. W. Edwards concludes that "... within various age and sex groups the pattern of distribution of fat is the same for all members of the group, and that changes in the total amount of fat stored in the body involve similar changes in every fat-storing cell." Thus if one can measure a fat fold accurately, then a good estimate of the quantity of fat in the body can be deduced from the thickness of one or more of these fat folds. Although there are many possible sources of error in measuring skinfold thickness, Edwards has found that the calipers used were the greatest cause of difficulty. He and his colleagues have described (*Brit. J. Nutrition* **9,** *133* (*1955*)) a suitable caliper, manufactured in England, which incorporates the following recommended features: (1) constant squeezing pressure over the whole jaw opening, range up to 50 mm.; (2) a squeezing pressure of 10 g. per square millimeter of jaw face; (3) a scale which allows readings to be made to the nearest 0.1 mm.

Edwards stresses that workers studying body composition should employ accurate instruments which have a constant and known squeezing pressure, and he urges that "... all who are interested will agree to a universally used pressure, in a precision-made caliper, which gives constant squeeze pressure, and reads accurately to 0.1 mm.'

Rapid Nutritional Responses

One of the major drawbacks to nutritional research using higher animals has been the length of time required to establish a deficiency. This difficulty has been due in large part to the stores of nutrients derived by the young animals from the mother's milk and to synthesis of nutrients such as vitamins by intestinal bacteria which are prevalent soon after birth. Use of microorganisms as nutritional test subjects or material has afforded only a partial solution to the basic problem.

Recently, R. J. Williams and B. G. DeBusk (*Proc. Nat. Acad. Sci.* **41,** *894* (*1955*)) have reported that the newly hatched chick, which is essentially bacteria-free and must obtain most of its nutrients from the immediate diet, can show nutritional responses within eighteen hours.

Newly hatched chicks were maintained on water only for the first day. They were then divided into groups which were placed on control diets, or experimental diets completely adequate except for omission of one suspected essential nutrient. After eighteen hours, food was withdrawn, and the weights of the groups were compared. If a significant difference did not appear, the diets were continued for one or two additional days and the chicks were again weighed. In all cases, weights and food efficiencies of the groups were compared statistically.

Nutrients the lack of which could be measured at eighteen hours were choline, vitamin A, thiamine, riboflavin, pantothenic acid, pyridoxine, vitamin B_{12}, biotin, lipoic acid, arginine, histidine, leucine and copper. After three days, deficiencies in lysine, tryptophan, cobalt, magnesium, methionine, glycine, cystine and polyunsaturated fatty acids were apparent. Only iron deficiency could not be produced, possibly because of adequate iron stores in the newly hatched chicks.

This technic should find uses in many studies. Quantitative data may be obtained for some nutrients as was done in the present case for pyridoxine. Tracing of possible unknown nutrients for the chick will be facilitated and speeded. Finally, it is possible that studies of this sort may be extended to other species, with resulting simplification of many nutritional experiments.

Food Consumption and Expenditure in Britain, 1952–1953

The British National Food Surveys for 1952 and 1953 ("Domestic Food Consumption and Expenditure 1952," Ibid., 1953, *Annual Report of the National Food Survey Committee, Her Majesty's Stationery Office, London (1954)*; *Ibid., 1955*) show that the good work of the 1950 and 1951 surveys (*Nutrition Reviews* **12,** *255 (1954)*) is being continued.

The 1952 survey, although roughly similar to that of 1951, incorporates some new features. For example, for the first time the survey covers not only all sections of the population, but operates throughout each month of the year. Such national coverage and continuity were made possible by the simplified field work already described ("Domestic Food Consumption and Expenditure 1951," *Annual Report of the National Food Survey Committee, Her Majesty's Stationery Office, London (1953)*; *Nutrition Reviews, loc. cit.*). Further, there are details concerning the effect of children in a family on food expenditure, a discussion on meals eaten outside the home, a section on the incidence of school meals and school milk, data on seasonal variations in the consumption of fruits and vegetables, and detailed statistical tables on the contribution of different foods to the nutrient content of the diet in households belonging to different social classes, and containing various numbers of children and adolescents.

The 1953 survey was conducted along similar lines; however, there is also a special section devoted to the Scottish diet, and a discussion of differences observed in 1952 and 1953 between the food consumption and expenditure of urban and rural households.

Apart from calcium and ascorbic acid, which remained constant, and vitamin D, which showed a 6 per cent fall, a general increase in the energy and nutritive value of the household diet was noted in 1953 compared with 1952, and provisional figures for 1954 indicate a continued rise. Comparison with allowances based on the recommendations of the British Medical Association (*British Medical Association; Report of Committee on Nutrition, 1950*) indicated that the average diet in 1953 was adequate throughout the entire year.

A comparison of the average nutritive value of urban and rural household diets in 1952 and 1953 indicated that the general improvement in the national diet between 1952 and 1953 was shared by both types of households. However, higher calcium and carbohydrate intakes were found in the rural households, and higher ascorbic acid intakes in the urban households. The greater consumption of bread and flour in rural households was mainly responsible for their higher calcium and carbohydrate intake.

In the 1952 survey it was concluded that the effect of family composition on domestic food expenditure and consumption was much more important than that of social class (defined in terms of the income of the head of the household), and the results of the 1953 survey again support this conclusion.

As regards the 1953 Scottish diet, some differences were noted compared with the English sample, but although there were "striking differences" in consumption patterns, comparisons of food intake with allowances based on the British Medical Association's recommendations showed strong similarity for all household types in Scotland and Great Britain for energy and all nutrients. In Scotland and the rest of Britain the diets of households with 3 or more children and with both children and adolescents were relatively low in protein and calcium; those of all households with children and adolescents were also slightly low in energy and riboflavin, and the diets of the old age pensioner households were low in iron.

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